ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Volume XLVI, Art 4. Pages 185–208

Editor
Roy Waldo Miner

RESPIRATION AND GERMINATION STUDIES OF SEEDS IN MOIST STORAGE

BY

Lela V. Barton

NEW YORK
Published by the Academy
August 18, 1945

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(Lyceum of Natural History, 1817–1876)

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[*] Awarded an Honorable Mention in the A. Cressy Morrison Prize Competition in 1944. Publication made possible through a grant from the income of the Nathaniel Lord Britton Fund.

[†] Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.

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INTRODUCTION

Numerous investigators have been concerned with the viability of seeds buried in the soil for long periods of time. Weed seeds especially have been the subject of many studies. No effort will be made here to give a complete review of the published literature on this subject. One experiment which was started by Beal will serve to indicate the remarkably long life span of certain weed seeds buried in the soil. Twenty sets of seeds were buried in 1879 in uncorked bottles in sandy soil 20 inches deep with the mouths tilted downward to avoid filling with water. *Amaranthus retroflexus* and *Rumex crispus* were among the seeds which remained viable for 40 years and some *Rumex crispus* seeds still germinated after 60 years' burial.[6] Since the seeds must have absorbed water soon after being placed in the soil and must have remained imbibed with water throughout the storage period, this length of life is noteworthy. Furthermore, these seeds are not usually considered dormant. That they do not develop a deep dormancy, when buried in the soil, is demonstrated by the fact that when the soil in an ordinary garden plot, for example, is disturbed by cultivation, many weed seedlings appear even if care has been taken to remove all such plants for several preceding years.

When seeds take up water preparatory to germination, their metabolic activities become greatly accelerated. It is evident that imbibed seeds could not remain viable very long in the soil unless definite curtailment of these activities take place. The present respiration studies were undertaken in an attempt to get a definite measure of one of the life activities of seeds which were imbibed with water but which were kept from germinating by placing at unfavorable temperatures. Studies were also made of the germination behavior of such seeds.

RESPIRATION

Materials and Methods

Seeds of *Amaranthus retroflexus* L. and *Rumex obtusifolius* L. were selected as experimental material, because some work had been done on these genera previously and because they were readily available for collection. Limited tests were also made using *Impatiens balsamina* L. seeds produced on the Institute grounds.

After collection the seeds were cleaned of all extraneous material and then counted in lots of 2500 each for *Amaranthus*, 1000 each for *Rumex*, and 100 each for *Impatiens*. A vacuum counter was used for *Amaranthus* seeds. Counted instead of weighed lots were chosen since it has been demonstrated that the number of individual embryos is important in determining respiration. Bailey and Gurjar[1] pointed out that shriveled wheat respired at a rate two or three times greater than did plump grains. This they attributed to the higher ratio of embryo weight to endosperm weight in shriveled grains as compared with that of the plump wheat. It has been demonstrated, however, that some endosperm tissues are capable of respiration.[15]

The counted lots of dry seeds were remarkably uniform in their weights. For example, weights of five lots of 2500 seeds each of *Amaranthus* were 0.94, 0.96, 0.95, 0.96, and 0.96 gram; of five lots of 1000 seeds each of *Rumex* 1.21, 1.22, 1.21, 1.23, and 1.25 grams; and of five lots of 100 seeds each of *Impatiens* 0.49, 0.51, 0.53, 0.45, and 0.57 gram. Variations in the weights of *Impatiens* were due to different sizes of seeds in the lot used, in spite of the fact that the largest and smallest seeds were screened out.

Since respiration measurements were to be made, care had to be taken to eliminate environmental factors which would alter gaseous exchange. The seeds were kept in constant temperature rooms large enough so that all respiration measurements could be made there. As will be shown later, imbibed seeds are very sensitive to temperature changes. Furthermore, mere mechanical disturbance of such seeds may bring about germination, in which case respiration would be greatly increased. This posed the problem of getting the seeds into a respiration chamber without disturbance. The appearance of mold on the seeds would also alter respiration, as might any sterilizing agent which could be used.

With all of these factors taken into consideration and after a considerable number of trials, the following method was adopted. The seeds were cleaned thoroughly by compressed air, but not washed. They were then placed on strips of moist glass wool. Glass wool was used because it tended to decrease the possibility of infection. It held moisture well, and was easy to handle. These strips of glass wool with the seeds were placed in sterile Petri dishes where they were kept until the respiration measurement was made. Constant temperatures used to hold moist seeds without germination were 20° C. for seeds of *Amaranthus* and *Impatiens*, and 30° C. for seeds of *Rumex*. They were examined at intervals and the moisture adjusted, using boiled tap water, and any seedlings removed. Also, careful check was kept on possible mold formation and any seed showing the slightest sign of infection was removed and discarded. A record of germinations and moldy discards was kept for each culture so that the exact number of seeds used for each respiration test was known.

For measurement of respiration, the glass wool strip containing the seeds was removed from the Petri dish and placed on a strip of paraffined cheesecloth of similar size. This was then inserted in the respiration tube as shown in PLATE 1. This tube was fitted at one end with a ground glass stopper the size of the internal bore of the tube and, at the other end, with a capillary stopcock and tubing to facilitate the removal of the gas sample. Usually, three to five tubes were set up for each test, since a culture could not be used for respiration measurements if seedlings or mold had appeared by the end of the respiration period. After several preliminary tests, a 48-hour respiration period was shown to be satisfactory.

The volume of gas in the respiration chamber varied according to the tube used and the size of the seeds, but was usually between 65 and 75 ml. Since this was 25 to 40 or more times the volume of the seeds being tested, respiration for 48 hours was permitted without unduly reducing the oxygen content or increasing the carbon dioxide content. At the same time, sufficient change occurred in the proportion of the two gases to give satisfactory measurements by gas analysis.

It is recognized that the moisture in the respiration tube might have absorbed some of the respired gases. Some measurements of oxygen and carbon dioxide in control tubes, with and without moist strips of glass wool, indicated a decrease in the former of 0.06 per cent CO_2 and 0.2 per cent O_2. This means then that all of the respiration readings for seeds were slightly lower than actual, but the constancy of the error permitted comparisons to be made.

The necessity of having a separate air control for each test was demonstrated early in the experimental work, for the air in the constant temperature rooms varied in oxygen and carbon dioxide content from time to time. Consequently, all of the results have been calculated using individual and separate controls for each test. The control tubes contained no moist glass wool.

At the end of the 48-hour period, the gas in the respiration tube was displaced by using a 22 per cent aqueous solution of sodium chloride recommended by Dennis and Nichols[7] as having minimum solubility for both oxygen and carbon dioxide. Analysis of the gas for both oxygen and carbon dioxide was made in an apparatus which is shown in PLATE 2. The apparatus is essentially that described by Haldane and Graham,[9] with certain adaptations and modifications designed by Dr. Norwood C. Thornton of this Institute to fit the special requirements of these experiments. There was a permanent mounting of the gas analysis and gas sampling and measuring burettes by a T connection, in order to take in samples and expel air from the gas analysis burette without loss of time or errors in handling of the original gas sample. The small absorption pipettes were filled with glass tubes to enlarge the absorption area of the liquid. The pipettes were connected to large reservoirs of the absorbing liquids in order that several consecutive determinations could be made without replenishing the absorbents. Fifteen per cent sodium hydroxide solution was used to remove carbon dioxide from gas samples. Oxygen was absorbed in an alkaline pyrogallate solution made by dissolving 26.25 grams pyrogallic acid in 175 ml. of 51 per cent potassium hydroxide. Solutions were changed when oxygen or carbon dioxide absorption became sluggish. The solutions lasted for at least 20 analyses. The volume of alkaline pyrogallate solution used in the apparatus was 175 ml. and that of sodium hydroxide between 60 and 70 ml.

The sample to be analyzed was displaced from the respiration tube A, PLATE 2, into measuring tube B by manipulation of reservoir L and by displacement of the gas from A with 22 per cent solution of sodium chloride following the removal of all air from the connecting tubes and the removal of the ground glass stopper of A under the surface of the sodium chloride solution. The sample was collected and measured in a gas burette B of 100 ml. capacity, with a graduation of 0.2 ml. This displacement by salt solution over to a tube containing mercury was effected with little or no carry over of water. If some water did go over, it was separated by settling on the mercury, so that an air sample free of water droplets could be drawn into the gas burette C. For gas analysis, 16 ml. were taken into this burette, which is graduated to 0.01 ml. and can be read accurately to 0.005 ml. The graduated part of this tube extends from 11.85 ml. to 16 ml. The remainder of the 16 ml. volume is supplied in the bulb at the top of the tube. Surrounding this gas burette is a water jacket and, attached to it by means of thick-walled rubber tubing, is a leveling vessel of mercury D, which is supported and manipulated by a pinion E. The gas sample was then ready for removal of carbon dioxide, which was accomplished by forcing the gas through the sodium hydroxide solution in absorption pipette F, which was filled with glass tubes to increase the absorbing surface. By raising and lowering the mercury through manipulation of vessel D, the gas was passed over the sodium hydroxide solution seven times and then a reading was taken on gas burette C. The difference between this reading and 16 ml. indicated the amount of carbon dioxide in the sample. After the reading was taken, the gas was passed over the hydroxide solution four more times and a second reading taken. If the two readings were identical, it was assumed that all of the carbon dioxide had been removed.

Absorption pipette F was then closed off and the same gas sample sent over the alkaline pyrogallate solution in absorption pipette G, also provided with glass tubes, and attached to reservoir H. The gas was sent over the pyrogallate solution ten times and then once again over the hydroxide solution to wash out the oxygen in the connecting tube. Then, sending it five additional times through the pyrogallate solution, followed by another washing out of the connecting tube, usually removed all the oxygen of the sample. After adjusting the pressure in the burette by using the hydroxide pipette, which had a scale attached on the capillary portion, and which had the other arm attached to the compensating tube K of the same design as the gas burette and also inserted in the water jacket, a reading was taken on gas burette C. A reading of the amount of oxygen in the sample could then be had by subtracting this value from the carbon dioxide reading already obtained. These values could be converted to percentages by multiplying the number of milliliters of carbon dioxide or oxygen by 6.25, as the total amount of the sample was 16 ml. Since the limit of reading on the gas burette was 0.005, the limit of accuracy in per cent was 0.03125.

At the beginning of each test, any pressure due to different levels of the pyrogallate solution and the water seal was released by opening a stopcock, between the two liquids, to the air. Also, the pressure on the sodium hydroxide solution and in the connected capillary compensator tube K was made the same as that of the atmosphere by opening the stopcock (above the letter K, PLATE 2) to the air. Before a final reading was taken, after any absorption, the gas was always passed through the alkali tube in which compensation had been made for the atmospheric pressure, and the level of the liquid in the tubing below the alkali stopcock had been brought to a predetermined level, set with the stopcock of the compensator tube open to the air before the gas analysis was started. Since the analyses were always made within a closed system, and pressure adjustments made before each test, barometric pressure effects may be disregarded in the results. Temperature effects were eliminated by the use of constant-temperature rooms.

The carbon dioxide and oxygen were always removed from the air within the gas-analysis apparatus before a new sample was taken, so that carbon dioxide- and oxygen-free air occupied the connecting tubes between G, F, and C. The connecting tubes between C, B, and A, as well as the small connecting tube on the respiration chamber itself (shown in PLATE 1), were filled with mercury before the gas was drawn from A.

Tests to determine the reproducibility of the respirometer readings were made. From a sample of air taken directly into the measuring burette, five tests of 16 ml. each were made. Duplicate readings from each test gave identical results for four of the five tests. The fifth test showed 0.02 per cent more carbon dioxide and 0.02 per cent less oxygen. This was within the limits of accuracy of the apparatus, which was 0.03125 per cent. Three tests on a sample of air taken from a respiration tube into the apparatus, with duplicate readings on each test, gave identical results for carbon dioxide and a difference of 0.02 per cent, in one case, for oxygen. A repetition of these tests on an air sample from another respiration tube gave identical amounts of carbon dioxide and oxygen in all cases. Also different operators obtained the same measurements from two samples of the same gas mixture.

Results and Discussion

Amaranthus retroflexus. Respiration measurements were begun, in the fall of 1941, on freshly-harvested seeds, but no reliable measurements were obtained until the respirometer modification was complete, so early measurements were meaningless. Some of the determinations made on these seeds at later periods will be given below.

Two seed collections were made in the fall of 1942. Collection A was obtained in Yonkers on September 28. Forty counted lots, of 2500 seeds each, were placed on moist glass wool in a room at 20° C. on October 6, 1942. Collection B was made at Emmaus, Pa., and shipped directly to Yonkers, arriving in this laboratory on October 1, 1942. Lots, as for Collection A, were placed on moist glass wool in a 20° C. room on October 13, 1942. The carbon dioxide output and the oxygen intake of these seeds after various lengths of time in moist storage are shown in TABLE 1. The gaseous exchange is expressed as milliliters of carbon dioxide evolved and oxygen absorbed by 2000 seeds in a 48-hour period.

It should be kept in mind that 20° C. is an inhibitive germination temperature for dry seeds only when they are freshly harvested. As they after-ripen in dry storage they become less exacting in their temperature requirements for germination. Also, crops and collections vary in the degree of dormancy exhibited by freshly-harvested seeds. Seldom does one find a collection in which all of the seeds are dormant. It was, therefore, difficult to get respiration measurements of dormant

TABLE 1

RESPIRATION OF SEEDS OF *AMARANTHUS RETROFLEXUS* HELD ON MOIST GLASS WOOL AT 20° C. FOR DIFFERENT LENGTHS OF TIME AFTER HARVEST

(Carbon Dioxide Given Off and Oxygen Taken up Expressed as Ml. per 2000 Seeds for a Period of 48 Hours)

Collection A				Collection B			
Days in moist storage	CO_2	O_2	CO_2/O_2	Days in moist storage	CO_2	O_2	CO_2/O_2
0	1.52	2.20	0.69	0	1.42	1.94	0.73
1*	1.74*	2.80*	0.62	1	1.36	1.92	0.71
2	1.36	2.20	0.62	2	1.18	1.76	0.67
8	0.90	1.48	0.61	8	0.66	1.16	0.57
16	0.62	0.98	0.63	16	0.04	1.18	0.03
35	0.48	0.94	0.51	32	0.62	1.00	0.62
67	0.34	0.76	0.45	63	0.38	0.84	0.45
134	0.32	0.66	0.48	134	0.26	0.60	0.43
218	0.40	0.80	0.50	211	0.40	0.62	0.65
385	0.18	0.68	0.26	375	0.24	0.50	0.48
564	0.18	0.42	0.43	551	0.12	0.98	0.12

* 3 small seedlings + 2 seeds split at end of respiratory period.

seeds for the one- and two-day moist storage periods. In spite of the fact that ten replicate tubes were set up for each seed collection for each of these periods, a reading for dormant seeds of collection A, after one day of storage, was not obtained. All of the tubes contained some seedlings. The best one had three small seedlings and two additional seeds split at the end of the respiration period. These data have high values as can be seen in the table. All of the other values were fairly consistent with the exception of the high oxygen uptake value for collection B, after 551 days of storage. Experimental procedure failed to reveal the cause of this.

It is evident that the respiration of seeds which imbibe water and still remain dormant was reduced soon after the water was absorbed. Between two and eight days, a rather sharp drop in respiratory activity was noted. This decrease continued with increased length of the storage period and probably was still to be seen after 551 and 564 days of moist storage. That the amounts of carbon dioxide given off and oxygen taken up will continue to diminish in these seeds is indicated by the data obtained from seeds of the 1941 crop. Respiratory activity of these seeds was measured after 375, 728, and 901 days, when the carbon dioxide given off per 2000 seeds, in a 48-hour period, was found to be 0.08, 0.02, and 0.04 ml. for the three periods respectively. The oxygen consumption for the same periods was 0.66, 0.36, and 0.28 ml. These seeds of the 1941 crop, stored 901 days, were still viable, as will be shown by germination tests below.

The respiratory quotient tended to decrease with increased time in moist storage (TABLE 1). The importance of the respiratory quotient as an index of what actually occurs in gaseous exchange of materials has been questioned by some workers. Many others, however, believe that certain conclusions can be drawn from the relative amounts of carbon dioxide given off and the oxygen taken up. Certainly, it can be said that the conditions under which the seeds were kept in this experiment affected not only the absolute, but also the relative rates of the two processes concerned (TABLE 1).

Sherman[13] conducted a study on the respiration of dormant seeds which included those of *Amaranthus retroflexus*. Measurements were made at intervals from 3 to 176 days after harvest. These seeds were held dry, however, until the time of testing, so that they did not remain dormant. She obtained variations in respiration in the different lots tested, but the respiratory quotient remained the same throughout. This value was somewhat higher (0.8) than that obtained with freshly-harvested seeds in the present study, but was still less than unity.

One of the chief determining factors in the value of the respiratory quotient is the chemical composition of the respiring materials. A chemical analysis of *Amaranthus retroflexus* seeds made by Woo[17] showed, that of the total constituents, 47.03 per cent are carbohydrates and 7.86 per cent lipins. From these data, one would expect a respiratory quotient approximating unity. On the contrary, the actual values found for freshly-harvested seeds was approximately 0.7 which is the respiratory quotient for the absolute oxidation of fats. At any one time, the respiratory quotient is related to the nature of the substance actually used at that time. Since the seeds contain very little fat, it is probably concentrated in the embryo. It is possible, then, that this oil supply is drawn upon immediately when the seed absorbs water and much oxygen used in its conversion to sugar. This would account for the initial low value of the respiratory quotient. Stiles and Leach,[14] reporting the respiration of germinating *Fagopyrum esculentum* seeds, said that the small reserve of fat in this seed was consumed at a very early stage in seedling development, in spite of the fact that the principal food reserve in the seed was starch.

Some tests were made on seeds of collection A, 1942 crop, in July 1944, to determine the respiration upon transfer to a 30° C. room. Respiration measurements were made after 1, 3, 6, 24, and 48 hours in a respiration tube. The seeds were placed in the respiration tube directly upon removal from 20° C. to the 30° C. room. The tubes and the paraffined cheesecloth had been kept at 30° C., but the seeds were left on the original moist glass wool which had been at 20° C. Respiratory activity could be measured, after one hour, at 30° C. The actual amounts of carbon dioxide given off and oxygen taken up were small, but the respiratory quotient was back up to 0.73 at that time. The amounts of gas respired increased with longer periods, but the quotient remained the same even after 24 hours in the respiration tube, when carbon dioxide and oxygen contents of the gas had reached 5 and 7 per cent, respectively, and when many seedlings had appeared in the tube. These results agree with those of Stiles and Leach[14] that the fat reserve is still being used at an early stage of seedling development. An extension of time in the respiration tube at 30° C. to 48 hours, the period used for all of the tests at 20° C., yielded 24 per cent carbon dioxide, and indicated the absorption of 20 per cent oxygen. The numerous seedlings were quite large at this stage and the considerable quantity of carbon dioxide, coupled with the exhaustion of the oxygen supply, doubtless affected the respiratory rate, so that these data can not be used for comparison. The increase after one hour at 30° C. in the respiratory quotient from the low value of the moist ungerminated seeds to the original value at the time of storage is not surprising in view of the sensitivity of these seeds to temperature changes reported below.

Brown[3] measured the respiration of dormant red oak acorns and found a lowered respiratory quotient with increased time in storage for three weeks at 2.5° C. This was due to an increased oxygen consumption, while carbon dioxide production remained the same. In the present tests, both carbon dioxide produced and oxygen absorbed were decreased with storage, but the proportions did not remain constant. This continued reduction in the value of the respiratory quotient with increased length of time that the seeds were held ungerminated at 20° C. might indicate the use of oxygen for purposes other than respiration. Such a storage of surplus oxygen is known to lead to the accumulation of acids and sugars. Also, it should be noted that a retention of carbon dioxide in the tissues would result in a lowered respiratory quotient. Whether such extremely low respiratory quotients can be sustained for long periods of time by seeds lying viable but dormant in the soil is a question. It should be emphasized that many variable factors which doubtless affect their life span in the soil were not present in the controlled experiments reported here. However, these studies indicate some of the fundamental changes which take place in seeds during moist storage and may serve as a beginning for further investigations.

Thornton and Denny,[16] working with gladiolus corms which had been held dormant in moist soil, determined that, in the phase when carbon dioxide was low, oxygen consumption was high. The volume of oxygen absorbed was usually two or three times as great as the volume of carbon dioxide given off. This is essentially the same relationship as reported here for dormant *Amaranthus retroflexus* seeds.

Approximately a ten-fold reduction in respiration has taken place in seeds of *Amaranthus retroflexus* by the end of a year of moist storage (TABLE 1). It is doubtful whether this reduction is sufficient to maintain viability for as long a period as 40 years.[6] Again, it should be pointed out that the conditions imposed upon the moist seeds in the experiments reported here were not strictly comparable to those which exist in natural soil burial. Physiological behavior of the seeds in the two environments must vary similarly. At a temperature of 15° C., for example, respiration would no doubt be lower at all times than at 20° C. and the imbibed seeds would probably still remain dormant.

Denny[8] reported that the respiration of gladiolus corms, which had been held in the dormant condition from October 1937 to May 1939, by placing the bulbs soon after harvest in moist soil and storing them at room temperature, exhibited very low respiration, not more than, and probably less than 10 per cent of the rate of non-dormant corms. This reduction was of the same magnitude as that reported here. Ota[12] also found that the carbon dioxide eliminated in the first 60 hours, after the upper seeds of *Xanthium* were placed on wet absorbent cotton in a respiratory chamber, was ten times that given off at the twentieth day. The upper seed of *Xanthium* exhibits delayed germination.

Catalase and oxidase activity, as well as respiratory intensity, has been shown by Crocker and Harrington[5] to be reduced in Johnson grass seeds held in a germinator at 20° C. for a year. The mechanism concerned in the reduction of respiration in dormant imbibed seeds is not known. That the seed coat membranes are responsible for it is not likely, since it has been shown for *Cucurbita pepo*[4] that, after prolonged contact with water, the permeability of the seed coat to gases increased.

Additional studies on respiration and other processes taking place within the seed are needed for explanation of the physiologic responses of these seeds.

Impatiens balsamina. From the data in TABLE 2, we see a trend toward reduction in respiratory activity, as well as in respiratory quotient, which is in general agreement with results from *Amaranthus* seeds. These seeds were of the 1941 crop and tests earlier than 28 days of moist storage were without value, because the respirometer was not reliable. It should be noted that these measurements were for 100 seeds and for a 24-hour period.

TABLE 2

RESPIRATION OF SEEDS OF *IMPATIENS BALSAMINA* HELD ON MOIST GLASS WOOL AT 20° C. FOR DIFFERENT LENGTHS OF TIME AFTER HARVEST

(*Carbon Dioxide Given Off and Oxygen Taken up Expressed as Ml. per 100 Seeds for a Period of 24 Hours*)

Days in moist storage	CO_2	O_2	CO_2/O_2
28	0.46	1.12	0.41
56	0.22	0.40	0.55
77	0.24	0.50	0.48
119	0.08	0.70	0.11
365	0.12	0.54	0.22

Rumex obtusifolius. Respiration measurements were made on seeds of both 1941 and 1942 lots of these seeds. The values obtained were not consistent, because mold formed on the seeds and could not be controlled. Sterilization with calcium hypochlorite failed to remedy the situation. Consequently, the actual amounts of oxygen used and carbon dioxide given off varied a great deal from test to test and actually increased somewhat with lengthened storage, due, no doubt, to the increased amount of mold. In spite of this, however, there was a general decline in the respiratory quotient with increased storage period from approximately 0.89, at the beginning, to 0.42 after 632 days, and 0.36 after 923 days of moist storage at 30° C.

GERMINATION

From time to time in the course of the experiments described above, special germination tests were conducted to determine the viability and germination behavior of seeds held moist and ungerminated. These tests involved special treatments which will be described below.

Results and Discussion

Amaranthus retroflexus. Seeds of these plants when freshly harvested, show a degree of dormancy which varies from year to year and, with seeds collected in various localities, any one year. When any freshly-harvested seed lot is placed under moisture conditions which favor germination, the temperature determines the number of seedlings obtained. This is shown in TABLE 3 for the two 1942 seed collections. These two lots were similar in their germination response. The best temperature for germination was constant 35° C. where 86 per cent seedlings were obtained for lot A and 73 per cent for lot B, immediately after collection. Furthermore, the temperature requirement was quite

TABLE 3

EFFECT OF TEMPERATURE ON THE GERMINATION OF FRESHLY-HARVESTED AND MOIST-STORED SEEDS OF *AMARANTHUS RETROFLEXUS* 1942 CROP

Germ. Temp., °C.	Per cent germination after moist storage at 20° C. for days			
	Collection A		Collection B	
	0	623	0	613
15	0	0	0	1
20	2	0	0	0
25	18	84	8	91
30	16	86	11	90
35	86	91	73	90
10 to 20*	0	21	0	12
10 to 30*	0	83	2	90
15 to 30*	1	84	6	83
20 to 30*	1	85	3	88

* Daily alternation. Cultures left for eight hours at the higher temperature and for sixteen hours at the lower temperature each day.

specific at that time. Some germination occurred at constant temperatures of 25° and 30° C., but the percentages here did not exceed 18 in either crop. Only occasional seedlings appeared over the rather wide range of other temperatures tried.

If these seeds were stored dry after harvest, a gradual change in their capacity for germination took place, so that, after two or three months, they germinated over a wide range of temperatures including 20° C. If, however, they were placed on a moist medium at 20° C. immediately following harvest, this after-ripening did not proceed but rather primary dormancy was maintained or a secondary dormancy developed. This resulted in a different pattern of germination behavior. These seeds were not all of equal dormancy, for a few germinated immediately, and others germinated at intervals with no apparent cause. Careful records of these germinations have been kept for one lot of 1941 seeds and for two lots produced in 1942. They have all exhibited a periodicity in germination. This periodicity has been remarkably uniform for the three lots. Within a period of two months after the seeds of 1942 crop collection A had been moistened and placed at 20° C., 4 to 32 seedlings had appeared in each of the cultures of 2500 seeds each (TABLE 4). Very few additional seedlings were obtained up to eight months of moist storage. Between eight and ten months many seeds germinated. The number varied greatly in the different cultures but germination occurred in all. In 1942 crop collection A, for example, four cultures produced less than 100 seedlings during that period; nine cultures produced from 148 to 339 seedlings; six cultures from 404 to 620 seedlings; and six cultures from 1043 to 1764 seedlings. Some increases were evident up to the fourteenth month of moist storage, but, by the

TABLE 4

AMARANTHUS RETROFLEXUS, 1942 CROP, COLLECTION A

(*Germination after Various Periods on Moist Glass Wool at 20° C., 2500 Seeds in Each Lot*)

Lot No.	Number of germinations at end of each 2-month period											Total germination
	2	4	6	8	10	12	14	16	18	20	22	
1	14	1	2	0	95	18	13	0	1	115	208	467
2	10	7	0	7	148	63	34	9	1	94	259	632
3	10	2	1	3	228	41	12	6	7	73	115	498
4	8	4	0	0	242	171	14	1	0	95	168	703
5	11	1	0	1	272	29	5	1	0	66	235	621
6	11	1	1	3	273	265	18	0	0	22	223	817
7	12	0	0	3	404	36	9	0	0	58	181	703
8	7	3	0	4	430	73	19	0	1	59	500	1096
9	32	0	0	14	474	27	18	0	1	41	182	789
10	10	3	1	5	524	41	24	3	0	28	246	885
11	13	1	1	4	1360	39	7	0	0	4	210	1639
12	12	1	0	5	1562	163	11	0	0	19	53	1826
13	11	2	3	2	1668	65	16	2	3	13	61	1846
14	11	3	3	6	1764	35	6	1	0	18	48	1895
15	13	2	0	2	29	199	28	2	2	49	936	1262
16	14	1	0	1	56	33	5	3	3	71	648	835
17	13	2	2	2	74	69	34	5	1	75	491	768
18	12	0	3	1	339	71	18	0	1	120	510	1075
19	9	5	0	3	1178	120	21	1	6	15	389	1747
20	10	0	0	1	215	19	10	1	1	25	1740	2022
21	11	2	0	4	255	25	35	1	2	65	1903	2303
22	8	3	1	3	284	36	21	2	1	27	1869	2255
23	11	0	0	2	612	12	8	1	0	16	1430	2092
24	6	1	0	4	620	97	33	6	0	25	1331	2123
25	4	2	0	2	1043	141	10	0	2	11	1074	2289

sixteenth month, only occasional seedlings were found. Most of the remaining seeds continued dormant until between the eighteenth and twentieth month of storage, when a second germination pulse was noted. Here, again, seedling production occurred over a period of approximately two months. The seed lots which had many germinations after ten months of storage yielded fewer seedlings in the second germination period, as was to be expected. Similarly, those with fewer germinations during the first period produced more seedlings during the second period.

The temperature of the 20° C. room, where these seeds were kept so that they could be examined and respiration tests made without temperature effects, could not be maintained during the summer months. Consequently, all of the seeds were transferred from the room to a 20° C. constant temperature chamber on June 29, 1943. This necessitated their removal from the oven to the laboratory for examination and watering. Since sensitivity to temperature change is characteristic of these seeds, it was thought that this may have brought about the increased germination. This seems unlikely, however, in view of the fact that the germination pulse did not come until July 26, in some cases, and was complete by August 2. The cultures were left in the 20° C. oven with weekly removal to the laboratory for examination until the first part of October with very few additional germinations. At this time, the cultures were replaced in the 20° C. room where the second germination pulse occurred after about 20 months. By the end of the 20-month storage period (early June, 1944), it was necessary to move the cultures again. At this time, however, a large room with a constant temperature of 20° C. was available. Lots 1 to 19 inclusive, TABLE 4, were transferred to this room so that it was not necessary to subject them to laboratory temperature for examination. Lots 20 to 25 inclusive were transferred to the constant temperature chamber, as in the summer of 1943, to determine more definitely the effect of the brief periods at laboratory temperatures. It will be seen that lots 20 to 25 all produced more seedlings, as represented by totals, than the other lots, but it was demonstrated conclusively that the second germination pulse did not depend on a change of temperature.

Under natural storage conditions in the soil, *Amaranthus retroflexus* seeds germinate before ten months. Bibbey[2] remarks that seedlings of this form appeared May 11 at Saskatoon, Saskatchewan, Canada, and Martin[11] listed these seeds among those that germinate late in spring in Iowa. He reported the same germination from *Amaranthus retroflexus* seeds held either wet or dry in refrigerators at 5° C. from before frost when they were harvested till early June when they were planted. If the temperature at time of planting was high, after-ripening would not have been a factor in this case.

Juby and Pheasant[10] used 25 sets of 100 seeds each of *Helianthemum guttatum* and found that they exhibited a constant intermittent germination. Only two germination maxima were involved, since germination was complete after the second one. Many other authors have observed that certain weed seeds are periodic in their germination, but very little definite experimentation has been done along this line. It seems, from the evidence at hand, that within any one seed lot varying degrees of dormancy, either primary or secondary, are found. This ensures the continuance of the species over a period of many years.

The germination capacity of the ungerminated seeds at various temperatures after 623 and 613 days of moist storage at 20° C. is shown in TABLE 3. It will be noted that such seeds have become less specific in their temperature requirement for germination than when they were freshly harvested. This would help to account for their germination under natural conditions. Their high germination capacity is in contrast to their low respiration as noted above.

In connection with temperature response, lots of seeds stored moist at 20° C. for approximately two years were removed and given 1, 2, 3, 4, 16, and 24 hours at 35° C., after which they were replaced at 20° C. Eighty-six, 75, and 90 per cent germination resulted after 4, 16, and 24 hours pretreatment respectively. The control lot, in this case, gave 15 per cent germination due, no doubt, to disturbance in transfer from the original seed lot to another Petri dish. The germination after two and three hours at 35° C. was only slightly less than that obtained after the four-hour period. One hour was not sufficient to induce much germination but produced more seedlings than the control.

Other experiments have been performed, using seeds stored moist at 20° C., to determine additional factors which might disturb the delicate dormancy equilibrium and bring about germination. Mechanical agitation of the soil in which the seeds are buried seems to bring about germination. We have found repeatedly that rubbing the seeds in the palm of the hand for 30 seconds was sufficient to induce approximately 50 per cent of them to germinate. Increasing the rubbing period to two minutes increased germination to 83 per cent in seeds held moist and dormant for one year. Similar results have been obtained with three crops stored two years. This suggests a coat restriction. Sulphuric acid treatment for two minutes was not as effective, but increased germination over that of the control.

Failure of certain *Helianthemum* seeds to germinate was due to an impermeable seed coat, according to Juby and Pheasant.[10] They attributed the intermittent germination to two factors: one, the physiological dimorphism of the seed ("hard" and "soft"), and the other, the wide range of variability of permeability of the seed coats.

The effect of drying the seeds for three hours, one day, and three days was noted. Such seeds returned to a moist medium at 20° C. gave 61, 59, and 73 per cent germination with the control at 0 per cent.

From these results, the difficulties involved in separating the several factors determining germination are apparent. Also the importance of a technique which would permit manipulation for respiration with a minimum of disturbance of the seeds is emphasized.

The seeds pictured in the respiration tube in PLATE 1 were removed from the 20° C. room and placed in the tube for photographing. They were out of the 20° C. room three and one-half hours, after which they were replaced. This was on July 13. On July 17, there were 1771 seedlings in this culture. In this case, it is impossible to determine whether mechanical disturbance (forceps were used to distribute the seeds evenly over the surface of the glass wool) or increased temperature brought about germination, since experiments have proved that either is effective. Tests have indicated that light does not favor germination, so could be eliminated as a factor.

Rumex obtusifolius. Although reliable respiration measurements could not be made on these seeds held ungerminated at 30° C. because of infection with mold, germination behavior was ascertained at intervals. Germination tests made on freshly-harvested seeds of both 1941 and 1942 crops indicated up to 98 per cent germination at a constant temperature of 15° C. or daily alternating temperatures of 10° to 20°, 10° to 30°, 15° to 30°, and 20° to 30° C. Slightly lower seedling production was obtained at 20° C. When the maintained temperature was as high as 25° C., germination was lowered to about 40 per cent, and no seedlings were produced at a constant temperature of 30° C.

Lots of 1000 seeds, each placed on moist glass wool at 30° C. immediately after harvest, showed continued primary dormancy or induced secondary dormancy, as was exhibited by *Amaranthus retroflexus* seeds at 20° C. Unlike *Amaranthus,* however, *Rumex* seeds showed no definite periodicity of germination under these conditions. There was some indication of increased germination after six and eight months of moist storage, but this effect was not marked. Rather, regular germination occurring throughout storage up to three years was characteristic. The number of seeds germinating was not large. In two years of storage, for example, representative lots had produced totals of 24, 41, 30, 74, and 101 seedlings out of 1000 seeds.

At intervals, these moist seeds were tested for germination capacity and for special treatments which might break their dormancy. Seeds of the 1941 crop still gave 97 per cent germination at a daily alternating temperature of 20° to 30° C. in June 1944. Special tests intended to bring about germination at the inhibiting temperature of 30° C. proved that the seed coat played an important rôle. Ungerminated year-old seeds germinated 96 and 90 per cent at 30° C., when the coats were broken or removed. Sulphuric acid treatment for two minutes induced 60 per cent germination. Seventy-four per cent of intact seeds transferred at 5° C. for three days germinated upon replacement at 30° C. A combination of sulphuric acid treatment and four days at 5° C. resulted in 93 per cent sprouting at 30° C. Some of these effects are shown in PLATE 3 A. It will also be seen (PLATE 3 B) that *Rumex* seeds after-ripen in dry storage. Here, the germination of seeds of the same original lot held dry and moist at 30° C. are compared.

Rubbing *Rumex* seeds was without effect on germination. Also, these seeds were not as sensitive to mechanical disturbance as were those of *Amaranthus.*

SUMMARY

Physiological studies were made on imbibed seeds which remain without germinating and viable over long periods of time. The test material consisted of seeds of *Amaranthus retroflexus, Impatiens balsamina,* and *Rumex obtusifolius.*

Gaseous exchange of *Amaranthus retroflexus* seeds, measured at intervals of from 0 to 901 days of moist storage at 20° C., showed at least a ten-fold reduction in respiration. The beginning of this reduction became apparent very early (after two days), and was definite after eight days in moist storage. Decreased respiration was also noted for *Impatiens balsamina* seeds held moist at 20° C. for 28 to 365 days. With increased length of time in moist storage, the respiratory quotient decreased.

Seeds of *Amaranthus retroflexus,* held in moist storage, showed a periodicity in germination which was apparently independent of external conditions. This indicated varying degrees of the primary dormancy or the induced secondary dormancy of the original lot of seeds. Moist *Amaranthus* seeds, held without germination at 20° C., could be induced to germinate at that same temperature by rubbing, by drying for three hours to three days, or by exposure to 35° C. for 12 to 24 hours. Germination also proceeded immediately after removal to higher constant or alternating temperatures.

Moist seeds of *Rumex obtusifolius,* held without germination at 30° C., could be made to germinate at this same temperature by removal of the coats, treatment with concentrated sulphuric acid for two minutes, or exposure to 5° C. for four days. Upon removal from 30° C. to lower constant temperatures or daily alternating temperatures, germination proceeded without further treatment.

Some of the implications of these responses are discussed.

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PLATES 1-3

Plate 1

Seeds of *Amaranthus retroflexus,* 1942 crop, Collection B, on strip of moist glass wool on paraffined cheesecloth in a respiration tube. Held moist at 20° C. from October 13, 1942 to July 13, 1944.

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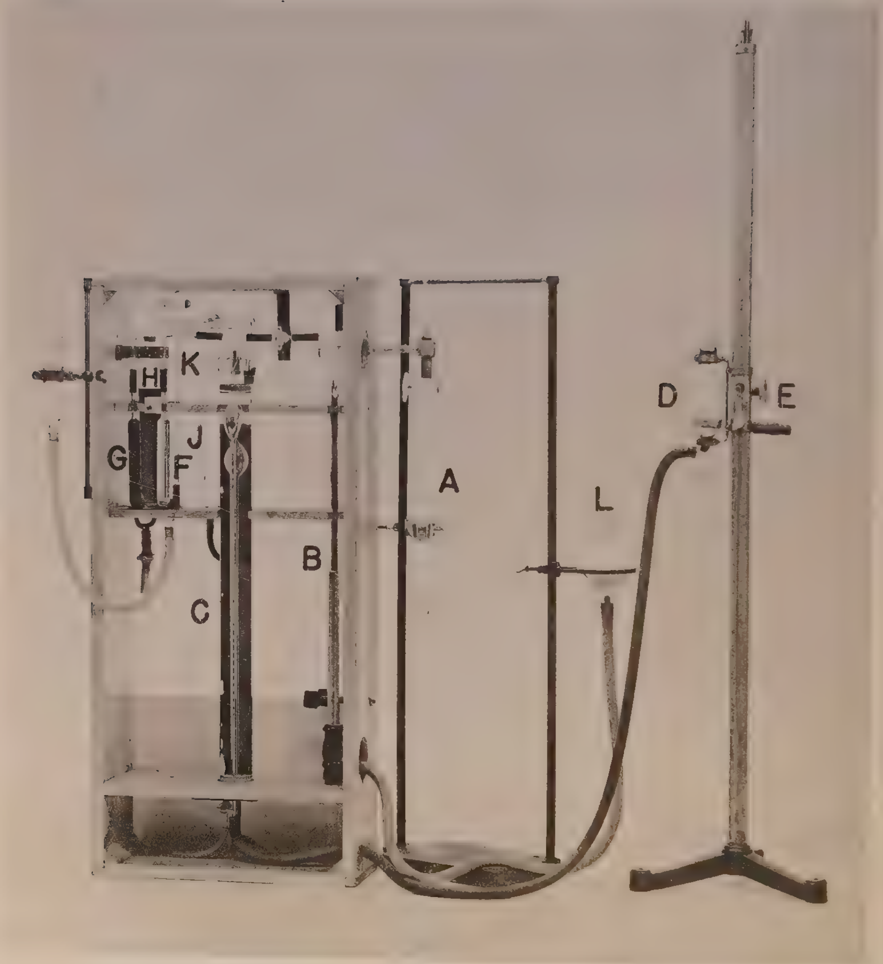

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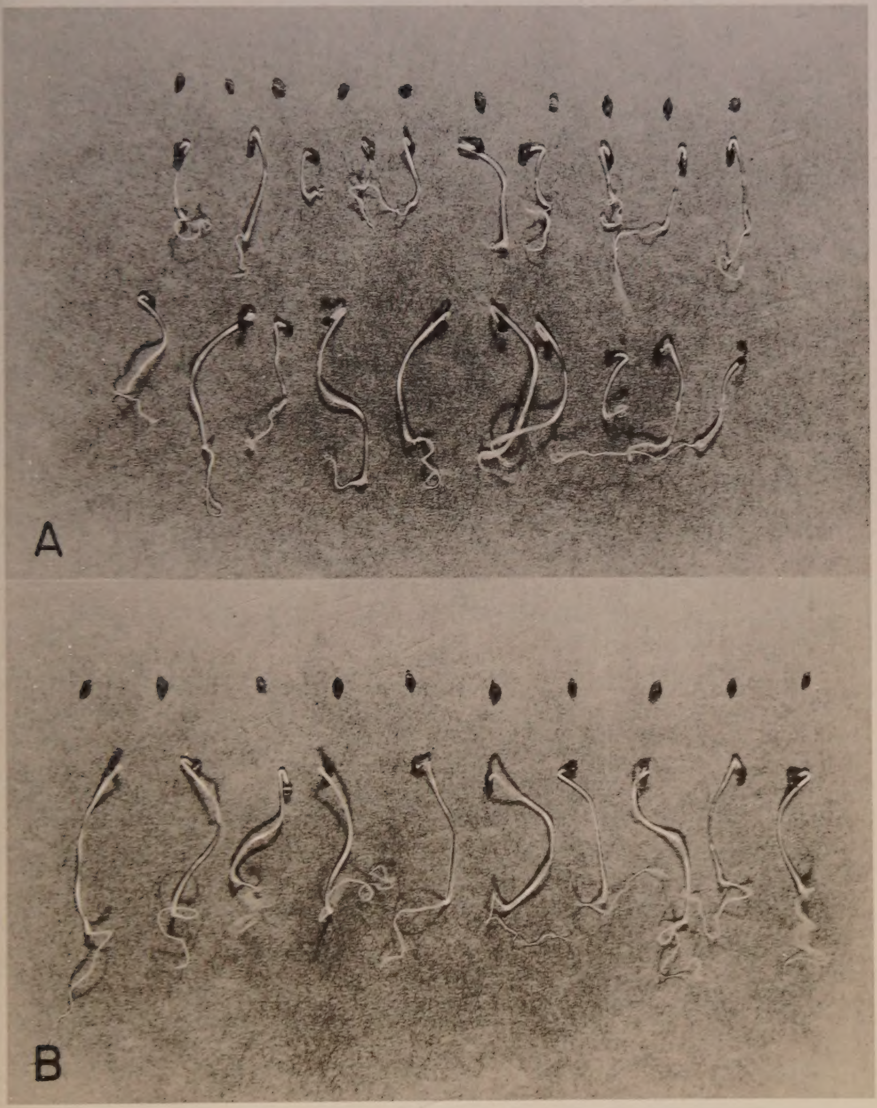
Plate 2

Modified respirometer for testing gaseous exchange of seeds.

PLATE 3

Rumex obtusifolius, 1941 crop. Photographed December 9, 1942. A. Seeds held moist at 30° C. from November 18, 1941 to November 30, 1942. Top row: germination temperature 30° C. (0%). Middle row: germination temperature 15° to 30° C. daily alternation (96%). Bottom row: germination temperature 30° C. after treatment for two minutes with concentrated sulphuric acid, followed by three days on moist filter paper at 5° C. (93%). B. Germination temperature 30° C. Top row: seeds held moist at 30° C. from November 18, 1941 to November 30, 1942 (0%). Bottom row: seeds held dry at 30° C. from November 18, 1941 to November 30, 1942 (81%).



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